Further Observation on the Role of Aneuploidy in Acute Leukemia*

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SUMMARY

Studies made before initiation of therapy and serial observations during consecutive relapses and intervening remissions in children with various types of leukemia are presented. Aneuploid stem lines were consistently demonstrable during the active stages of the disease, showing stability and occasionally traceable secondary evolution of modal numbers. The findings constitute further evidence for an association between neoplastic transformation and gross chromosomal abnormalities.

Aneuploidy of blood cell precursors is now widely recognized as a common phenomenon in acute human leukemias (4, 9, 11), but its significance, like that of the abnormal chromosome patterns seen in a variety of other neoplastic diseases of man and other species (5), is still under discussion. Is it, as has been suggested (1, 7), a purely secondary manifestation of neoplasia, reflecting only the spontaneous or therapeutically induced deterioration of the replicating mechanism in a population of cells inherently prone to random mitotic accidents? Or is an aneuploid constitution an integral part of the mutational change initiated by the unknown etiologic agent (or agents), a manifestation at the gross chromosomal level of the basic disturbance affecting the ancestral cell of a malignant clone, and a persistent attribute of its descendants? Although susceptible to other interpretations, the abundantly documented fact that the chromosome number in the aberrant metaphases generally shows a sharply modal distribution favors the second of these alternatives. This view does not preclude the possibility of further derangements of the mitotic apparatus, but it does imply the existence of relatively stable aneuploid stem lines which should be demonstrable in the earliest stages of the disease open to inspection.

In a previous study of various types of childhood leukemia virtually perfect correlation was obtained (9) between the cytologic status of the bone marrow and the modal karyotype determined by a "direct" method. Aneuploidy was consistently found whenever leukemic infiltration was present, whether at the time of the original diagnosis or at any time thereafter, up to 8 years after the first manifestations of the disease. During remission, on the other hand, the mode was always diploid (except in the case of children with the Down's syndrome who exhibited the expected mode of 47 with the typical trisomy 21), irrespective of the time elapsed since diagnosis and irrespective of the therapeutic agents previously or currently used. The aneuploid cells seen during the active stages of the disease invariably showed clear-cut modal distribution of karyotypes. The modal number varied considerably from case to case, but supernumerary chromosomes generally fell into the same groups, C, D, and G. The present report supplements these observations with respect to the prevalence of modal aneuploidy in the earliest clinical stages of childhood leukemia and the essential stability of the aneuploid stem lines. The further evolution of secondary karyotypes was observed in two cases.

CASE MATERIAL AND METHODS

All chromosome studies were performed by a previously described direct method (10) on bone marrow specimens incubated for approximately 1 hour. Aliquots were stained in the usual manner for cytologic examination. With the exception of a 17-year-old girl whose disease had first been diagnosed at the age of 9 years and had recently entered into relapse after an uninterrupted remission of more than 6 years, the patients were children ranging in age from 2 to 7 years. In all but one case the cytologic diagnosis was "stem cell" (lymphoblastic) leukemia. Immediately upon diagnosis each patient was placed on a regimen designated as "composite cyclic therapy" (15) and consisting in the initial administration of steroids in combination with 6-mercaptopurine, followed by the cyclic use of the latter drug in rotation with methotrexate. In subsequent relapses steroids were resumed in combination with whichever of the two antimetabolites remained

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effective, and ultimately variable therapy was employed—including the use of cyclophosphamide, vincristine, and massive doses of methotrexate given intravenously.

RESULTS

The previous report (9) had included seven cases in which pretreatment specimens adequate for chromosome studies had been obtained. Since then six additional patients could be studied at the time of diagnosis, bringing the series to the significant total of thirteen cases, all showing initial aneuploid karyotypes of modal distribution as summarized in Table 1. These findings extend the observations of Sandberg *et al.* (11), who could find no difference in the incidence of aneuploidy between untreated and treated leukemias, and are compatible with the postulate that aneuploidy, when present, is an inherent characteristic of the neoplastic cells.

In eight cases it was possible to make serial observations during repeated relapses and intervening remissions, and in some instances during sustained relapse. One of these requires separate consideration, the remainder are summarized in Table 2, which shows the variation in modal numbers from case to case, ranging in this series from 47 to 65. Clustering about the mode was always distinct but especially striking in those cases in which the modal number was very much higher than 46, as in case No. 20 with a hypotetraploid karyotype of 65 chromosomes in which no intervening numbers were found over a considerable range. This suggested that cells with chromosome numbers approaching the modal number belonged to one and the same stem line, the scatter reflecting as it were secondary mitotic accidents to be expected in an unbalanced mitotic system.

During remission the normal diploid mode of 46 was invariably restored, regardless of the duration of the disease or the therapy employed. Of interest was the occasional finding of single aneuploid cells with chromosome numbers identical with or close to, the modal number of the aneuploid cells found during the preceding relapse. Although aneuploidy is known to occur to a slight degree in nonleukemic bone marrows (11), the relationship of the aberrant karyotypes seen during "remission" (as defined by ordinary cytologic criteria) and the modal number prevalent during relapse suggested that the aneuploid stem line was not completely suppressed. Such findings may therefore provide a more sensitive index of the true extent of a remission than heretofore available. The converse did not hold true, however. As might be expected in view of the relatively small number of metaphases examined, failure to find aneuploid cells during an apparently complete remission could not be taken as prooof of total suppression of the leukemic stem line; and indeed, in two patients showing a completely diploid pattern during remission, relapse was observed soon afterwards.

In the second (or third) relapses studied, the originally observed aneuploid karyotype always re-emerged after remissions of varying length up to 4 months. The modal numbers of the aneuploid stem lines generally did not change during sustained relapses for periods of observation up to 8 months. A bimodal pattern with a minor peak of 46 was frequently noted, as might be expected as long as the bone marrow contains any residual normal cells. Obviously, the determination of the chromosomal mode must be based on the count of a substantial number of metaphases.

TABLE 1

MODAL NUMBERS OF ANEUPLOID CELLS FOUND IN THIRTEEN PATIENTS BEFORE THERAPY

Case	Type of leukemia	Modal chromosome number in bone marrow
1	Acute stem cell	55
2	Acute stem cell	49
3	Acute stem cell	61
4	Acute stem cell	48
5	Acute stem cell	55
6	Acute stem cell	52
7	Acute stem cell	48
8	Acute stem cell	60
9	Acute granulocytic	45
10	Acute granulocytic	45
11	Acute stem cell	46
12	Acute stem cell	50
13	Acute stem cell	54

Of particular interest were two cases in which the evolution of a second modal karyotype from the original aneuploid stem line could be followed. One of these (case No. 18, Table 2) happened to show a distinct marker chromosome which permitted the identification of the stem line regardless of the chromosome numbers and which was not present in the diploid cells forming a minor population during relapse and replacing the aneuploid cells during an intervening remission. At the time of the first relapse studied a single aneuploid stem line with a sharply defined mode of 56 was found. The No. 2 chromosome of this line was a marker chromosome with abnormally long arms. During the following remission this cell line was completely suppressed. In the ensuing second relapse the original stem line with 56 chromosomes including the marker re-emerged, but in addition an equal number of cells with 57 chromosomes was noted. This one line likewise exhibited the marker chromosome No. 2 as an indication of its origin from the original stem line, most likely as the result of endoreduplication of a single chromosome located in the C group (Figs. 1–3).

The second case (No. 21) was that of the 17-year-old girl whose bone marrow when first examined for its chromosomal pattern was in relapse and showed a modal karyotype of 47, including a minute chromosome and an extra chromosome in the C group (Fig. 4). The same modal number and marker chromosomes were found on several occasions during the subsequent sustained relapse. In a later specimen examined for its cytologic features a striking change was noted—the appearance of veritable giant cells no longer resembling the previously typical lymphoblasts of earlier specimens (Fig. 5). At this point numerous polyploid metaphases, including tetraploid cells showing the phenomenon of endoreduplication and the minute chromosome, were found in the marrow preparations (Fig. 6). Again, the evolution of a polyploid from a hyper-

TABLE 2

DISTRIBUTION OF CHROMOSOME NUMBERS IN BONE MARROW CELLS

CASE NO.	TIME AFTER DIAGNOSIS (MONTHS)	CLINICAL STATUS	CURRENT THERAPY	CHROMOSOME NUMBER																							TOTAL NO. CELLS COUNTED
				<46	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	
1	0	Relapse	Pred., 6-MP	3	8	34	2	1	—																		48
	1	Remission	Pred., 6-MP	5	11	—																					16
	2	Remission	Pred., 6-MP	2	24	—																					26
	5	Relapse	Pred., 6-MP	—	2	17	1	—																			20
	11	Relapse	Pred., cytox.	4	9	28	1	—																			42
14	14	Relapse	Pred., 6-MP	3	6	1	1	—	18	1	1	—															31
	17	Remission	6-MP	5	21	—	1	—																			27
	19	Part. rel.	MTX	3	19	1	—	2	3	—																	28
	22	Part. rel.	Cytox.	5	23	—	1	1	6	1	—																37
	25	Remission	Cytox.	4	19	—																					23
15	14	Relapse	MTX	5	4	1	—	5	4	12	—	2	1	1													35
	16	Remission	MTX	4	29	—																					33
	18	Part. rel.	Vincr.	2	13	—	1	1	3	6	1	1	—														28
	19	Part. rel.	Vincr.	4	17	—	1	4	9	24	2	1	1	—													63
	21	Relapse	Pred.	4	8	1	2	2	4	16	1	—	—	1	1	—											40
16	1	Relapse	Pred., MTX	5	3	—	—	—	2	4	3	4	15	2	—												38
	1	Remission	Pred., MTX	3	15	—	—	—	—																		18
	5	Relapse	Cytox.	2	6	—	1	—	—	2	1	3	16	4	1	—											36
	6	Remission	Cytox.	3	19	—	—	1	—																		23
	7	Remission	Cytox.	5	21	—	—	—	—	—	—	—	2	—													28
	9	Remission	Cytox.	4	31	—																					35
	9	Remission	Cytox.	3	5	—	1	—	1	—	2	5	13	—	1	—											31
17*	19	Relapse	Cytox.	1	—	3	—	2	—	2	—	1	—	21	4	—	—	1	1	—							36
	21	Remission	Pred., 6-MP	4	1	14	—	—	—	—	—	1	—														20
	23	Remission	MTX	3	—	42	—	—	—	—	—	1	4	—													50
	24	Relapse	Pred., 6-MP	1	1	5	—	—	1	—	2	—	3	19	2	1	—	—	1	—							36
18	25	Remission	MTX	5	20	—																					25
	28	Relapse	Pred., Cytox.	2	5	—	—	1	1	—	4	6	4	9	17	—	1	—									50
	30	Relapse	Pred., Cytox.	3	7	1	—	—	2	1	3	2	2	9	12	3	1	—									46
	31	Remission	MTX	5	19	—	—	—	—																		24
	32	Relapse	MTX	1	6	—	—	—	—	—	1	—	2	7	15	15	2	—	—	1	1	—					51
	33	Relapse	MTX	2	14	—	—	—	—	—	1	—	—	4	5	9	4	3	1	—							43
19	15	Relapse	MTX	7	3	—	—	1	—	—	1	—	1	1	—	—	3	1	6	21	5	1	1	—			52
	19	Relapse	Cytox.	6	5	—	—	1	1	2	—	—	1	2	—	—	3	3	4	19	4	1	—				52
	20	Remission	Cytox.	3	31	—	—																				34
	22	Relapse	Vincr.	5	9	—	—	—	—	—	—	—	1	—	—	—	1	1	9	14	1	1	—	1	—		43
	24	Remission	Vincr.	1	21	—																					22
	25	Relapse	Pred., Cytox.	—	8	—	—	—	—	—	—	—	—	—	—	3	5	5	5	19	2	2	1	—			50
	26	Relapse	Pred., Cytox.	2	8	—	—	—	—	—	1	—	—	—	—	—	3	—	1	2	—	2	—				19

20	24	Relapse	Pred., MTX	6	5	—	—	1	—	—	—	—	—	—	—	—	—	—	—	2	—	4	3	29	1	1	1	53
	25	Remission	Pred., MTX	2	13	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	18
	26	Relapse	MTX	—	2	1	—	1	—	—	—	—	—	—	—	—	—	—	4	—	1	3	6	20	2	—	—	40
	29	Part. rel.	Pred., Cytox.	3	41	—	1	—	1	—	—	—	—	—	—	—	—	1	2	2	—	4	2	8	1	1	—	57
	30	Relapse	Pred., Cytox.	3	11	1	—	—	—	—	1	—	—	—	—	—	—	—	2	2	1	4	4	17	1	—	—	47
	31	Relapse	Cytox.	4	7	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	2	12	—	1	—	28
	33	Relapse	Pred., 6-MP	2	4	—	2	—	—	2	—	—	—	—	—	—	—	—	4	1	2	4	1	14	1	2	—	39

* Down's Syndrome, Trisomy 21.
Pred. = prednisone; 6-MP = 6-mercaptopurine; Cytox. = Cytoxan (tryclophosphamide); MTX = methotrexate; Vincr. = Vincristine.

diploid stem line illustrated the continuity in the behavior of the abnormal karyotype, as opposed to a random accumulation of mitotic accidents.

DISCUSSION

The observations described conclusively support the thesis (5, 9, 11) that the aneuploid cells dominant in the active stages of leukemia represent true stem lines. As such they meet the criteria required by the hypothesis that a change in the chromosomal constitution of the malignant cell is one of the basic alterations in acute leukemia. These criteria are: (*a*) modal distribution of the abnormal karyotype, (*b*) presence of the abnormal karyotype in the earliest phase of the disease that can be investigated, and at any time thereafter in which the marrow shows leukemic infiltration, (*c*) stability over long periods of time, (*d*) suppression during remission, and (*e*) restoration of the identical (or a closely related mode) in subsequent relapse.

As pointed out by Sandberg *et al.* (11), the fact that aneuploidy has not been demonstrable in all cases of acute leukemia studied thus far does not detract from the intimate relationship between neoplasia and gross chromosomal abnormalities. Failure to demonstrate an aberrant stem line may be the result of relying too heavily on the determination of a single modal number which may be diploid even in the presence of absolutely increased numbers of aneuploid cells. This could be true if conditions *in vivo* were to favor mitosis of residual normal cells, since every cell population in acute leukemia must represent a mosaic. The fallacy of relying on the modal number alone under *in vitro* conditions has been abundantly shown. Some of the leukemias studied by Sandberg *et al.* with the direct method showed diploid modes yet a significant increase of frank aneuploid metaphases. Another more obvious source of error is the lack of a sufficiently large sample of cells. Thus, two of the cases of myeloblastic leukemia in adults reported by these authors had the "diploid" mode of 46, but the total number of countable metaphases was five and three, respectively. Finally, as stated by Hauschka (5), pseudodiploidy may simulate a normal chromosomal constitution, a fact which requires painstaking analysis of apparently diploid cell populations with respect to individual chromosomes and has posed great difficulties in the evaluation of murine karyotypes because of the resemblance of the chromosomes to one another in that species.

Nevertheless aneuploidy has been found in a high percentage of certain types of mouse leukemias (3, 6, 12, 13), and recently the consistency of a stem line with an aneuploidy and a marker chromosome in mouse leukemia induced by irradiation was shown in passage experiments with spleen cells, spleen extracts, and cell-free plasma (14). These observations, like the findings reported here in acute human leukemia, are difficult to explain on the assumption that chromosomal aberrations represent accidental events in leukemogenesis. In order to assign them a significant role it is not necessary, however, that abnormalities at the gross chromosomal level be demonstrable in every case.

The suggestion that the modal distribution of the aneuploid chromosome numbers reflects selection from originally random populations of cells because of some hypothetical growth advantage for one particular constitution (7) implies that the growth of the remaining malignant cells is restricted by factors inherent in the mitotic apparatus which would thus in effect impose its pattern on the neoplasia. Although this possibility cannot be excluded with the information at hand, it is more difficult to reconcile it with the observations than is that of a primary association between induction of neoplastic properties and chromosomal abnormalities.

The fact that the modal numbers in the human leukemias vary widely from case to case does not detract from the possible specificity of the initial trigger mechanism, be it viral, physical, or chemical in nature, although the possibility of a relationship between chromosome pattern and etiology has been considered (5). Rather, the variability is in keeping with the evidence that relatively gross structures in the mitotic apparatus are involved. It is not to be expected that the causative agents should produce identical effects at precisely the same locus in every case or in every cell, as illustrated by the variable chromosome breaks obtainable with viruses (8) or with x-radiation (2). This is not to imply that the primary effect in leukemia is necessarily restricted to the chromosomes themselves. Damage to the spindle elements or other cytoplasmic components might conceivably be involved. Any of these modalities can be reconciled with the observed correlations between karyologic and hematologic status in acute leukemia.

REFERENCES

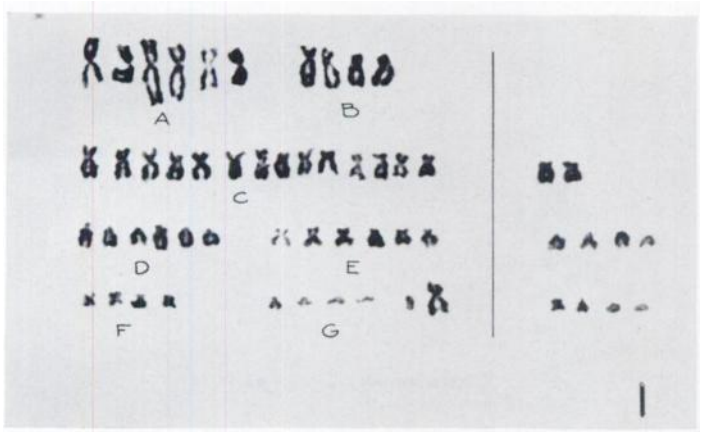
1. Bayreuther, K. Chromosomes in Primary Neoplastic Growth. Nature, **186**:2–9, 1960.
2. Bender, M. A., and Gooch, P. C. Chromosome Aberrations in Irradiated Human Subjects. Rad. Res., **16**:44–53, 1962.
3. Ford, C. E.; Hamerton, J. L.; and Mole, R. H. Chromosomal Changes in Primary and Transplanted Reticular Neoplasms of the Mouse. J. Cell. and Comp. Physiol., **52**(Suppl. 1): 235–69, 1958.
4. Gunz, F. W., and Fitzgerald, P. H. Chromosomes and Leukemia (Editorial). Blood, **23**: 394–400, 1964.
5. Hauschka, T. S. Chromosome Patterns in Primary Neoplasia. Exp. Cell. Res. (Suppl.), **9**:86–98, 1963.
6. Kurita, Y., and Yosida, K. H. Chromosomal Alteration and the Development of Tumors. Gann, **52**:257–64, 1961.
7. New England Journal of Medicine, Editorial. Chromosome Changes in Leukemia. New Eng. J. Med., **270**:635–36, 1964.
8. Nichols, W. W.; Levan, A.; Hall, B.; and Östergren, G. Measles-Associated Chromosome Breakage. Preliminary Communication. Hereditas, **48**:367–70, 1962.
9. Reisman, L. E.; Mitani, M.; and Zuelzer, W. W. Chromosome Studies in Leukemia. I. Evidence for the Origin of Leukemic Stem Lines from Aneuploid Mutants. New Eng. J. Med., **270**:591–97, 1964.
10. Reisman, L. E., and Trujillo, J. M. Chronic Granulocytic Leukemia of Childhood: Clinical and Cytogenetic Studies. J. Pediat., **62**: 710–23, 1963.
11. Sandberg, A. A.; Ishihara, T.; Crosswhite, L. H.; and Hauschka, T. S. Chromosomal Dichotomy in Blood and Marrow of Acute Leukemia. Cancer Res., **22**:748–56, 1962.
12. Stich, H. F. Chromosomes of Tumor Cells. I. Murine Leukemias Induced by One or Two Injections of 7,12-Dimethylbenz (a) anthracene. J. Natl. Cancer Inst., **25**:649–62, 1960.
13. Wakonig, R., and Stich, H. F. Chromosomes in Primary and Transplanted Cells of Leukemias of AKR Mice. J. Natl. Cancer Inst., **25**:295–305, 1960.
14. Wald, N.; Upton, A. C.; and Jenkins, V. K. The Consistent Occurrence of an Extra and a Marker Chromosome in Two Passaged Mouse Granulocytic Leukemias. Blood, **22**:817–18, 1963.
15. Zuelzer, W. W. Implications of Long-Term Survival in Acute Stem Cell Leukemia of Childhood Treated with Composite Cyclic Therapy. Blood (in press).

Fig. 1.—Karyotype of cell with 56 chromosomes from bone marrow of Case No. 13. The chromosomes are arranged according to the Denver-Patau system, and the extra chromosomes are to the right of the dark line. The karyotype also illustrates the abnormal No. 2 chromosome in the A group.

Fig. 2.—Karyotype of cell with diploid complement of 46 chromosomes from bone marrow cell of Case No. 18 during remission. The No. 2 "marker" chromosome is not present.

Fig. 3.—Karyotype of bone marrow cell from Case No. 18 during subsequent relapse, demonstrating stem line of 57 chromosomes, including the long-armed No. 2 chromosome and an additional C group chromosome.

Fig. 4.—Karyotype of aneuploid stem-line cell from bone marrow (Case No. 21) in relapse. Arrows indicate "marker" chromosomes, a minute unit placed in the G group and an extra chromosome in the C group.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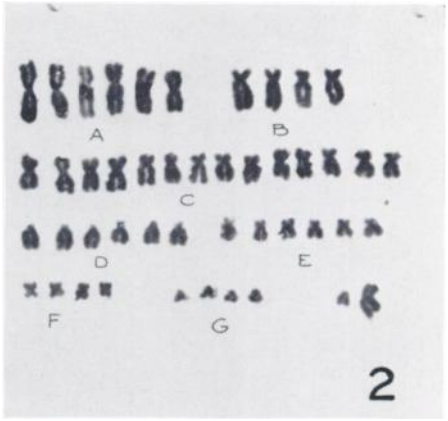
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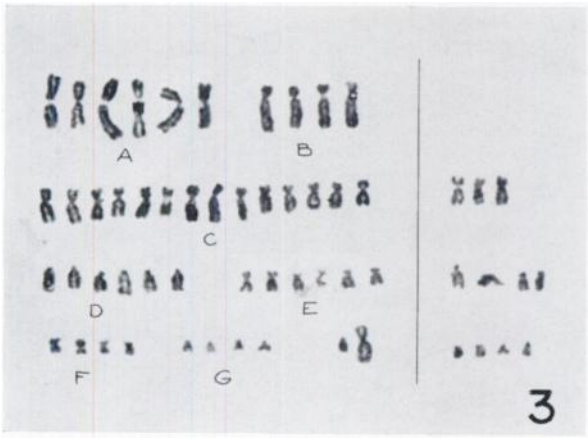
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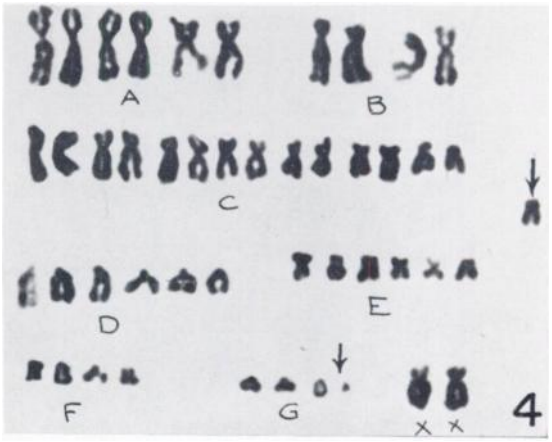
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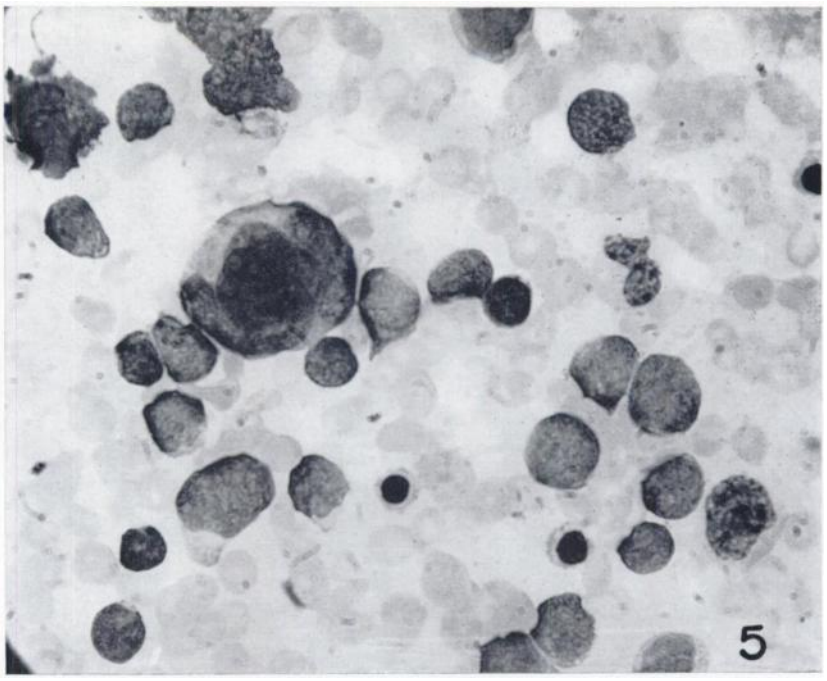
FIG. 5.—Photomicrograph of representative area of bone marrow in patient showing several giant "blast cell" forms in addition to the typical lymphoblasts.

FIG. 6.—Tetraploid metaphase from bone marrow of the same patient (No. 21) in terminal relapse, with 94 chromosomes. Arrows indicate the minute chromosomes. Metaphase also demonstrates the phenomenon of endoreduplication.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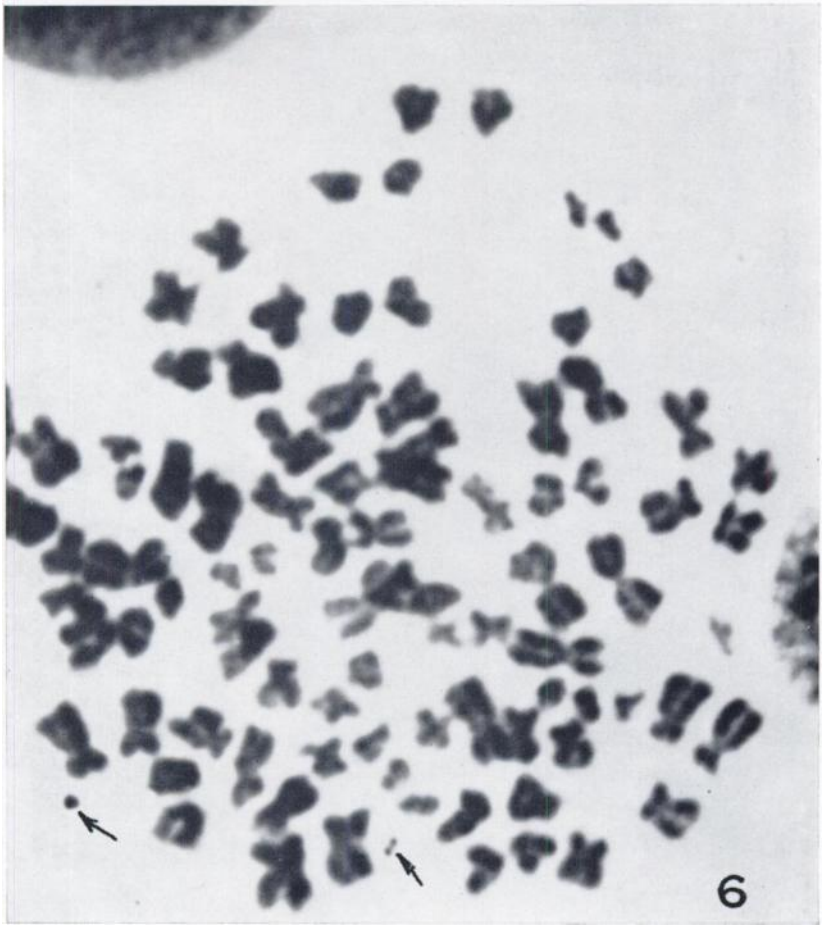
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